```
    ecoRI
  1 GAATTCAACT TCTCCATACT TTGGATAAGG AAATACAGAC ATGAAAAATC TCATTGCTGA GTTGTTATTT AAGCTTGCCC AAAAAGAAGA AGAGTCGAAT
    CTTAAGTTGA AGAGGTATGA AACCTATTCC TTTATGTCTG TACTTTTTAG AGTAACGACT CAACAATAAA TTCGAACGGG TTTTTCTTCT TCTCAGCTTA

101 GAACTGTGTG CGCAGGTAGA AGCTTTGGAG ATTATCGTCA CTGCAATGCT TCGCAATATG GCGCAAAATG ACCAACAGCG GTTGATTGAT CAGGTAGAGG
    CTTGACACAC GCGTCCATCT TCGAAACCTC TAATAGCAGT GACGTTACGA AGCGTTATAC CGCGTTTTAC TGGTTGTCGC CAACTAACTA GTCCATCTCC

201 GGGCGCTGTA CGAGGTAAAG CCCGATGCCA GCATTCCTGA CGACGATACG GAGCTGCTGC GCGATTACGT AAAGAAGTTA TTGAAGCATC CTCGTCAGTA
    CCCGCGACAT GCTCCATTTC GGGCTACGGT CGTAAGGACT GCTGCTATGC CTCGACGACG CGCTAATGCA TTTCTTCAAT AACTTCGTAG GAGCAGTCAT

301 AAAAGTTAAT CTTTTCAACA GCTGTCATAA AGTTGTCACG GCCGAGACTT ATAGTCGCTT TGTTTTTATT TTTTAATGTA TTTGTAACTA GTACGCAAGT
    TTTTCAATTA GAAAAGTTGT CGACAGTATT TCAACAGTGC CGGCTCTGAA TATCAGCGAA ACAAAAATAA AAAATTACAT AAACATTGAT CATGCGTTCA

              Trp SD   xbaI       STII SD
401 TCACGTAAAA AGGGTATCTA GAGGTTGAGG TGATTTT    ATG AAA AAG AAT ATC GCA TTT CTT CTT GCA TCT ATG TTC GTT TTT TCT
    AGTGCATTTT TCCCATAGAT CTCCAACTCC ACTAAAA    TAC TTT TTC TTA TAG CGT AAA GAA GAA CGT AGA TAC AAG CAA AAA AGA
                                               Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe Ser
  1

486 ATT GCT ACA AAT GCC TAT GCA (SEQ ID NO: 13)
    TAA CGA TGT TTA CGG ATA CGT
 17 Ile Ala Thr Asn Ala Tyr Ala (SEQ ID NO: 14)
```

FIG. 1

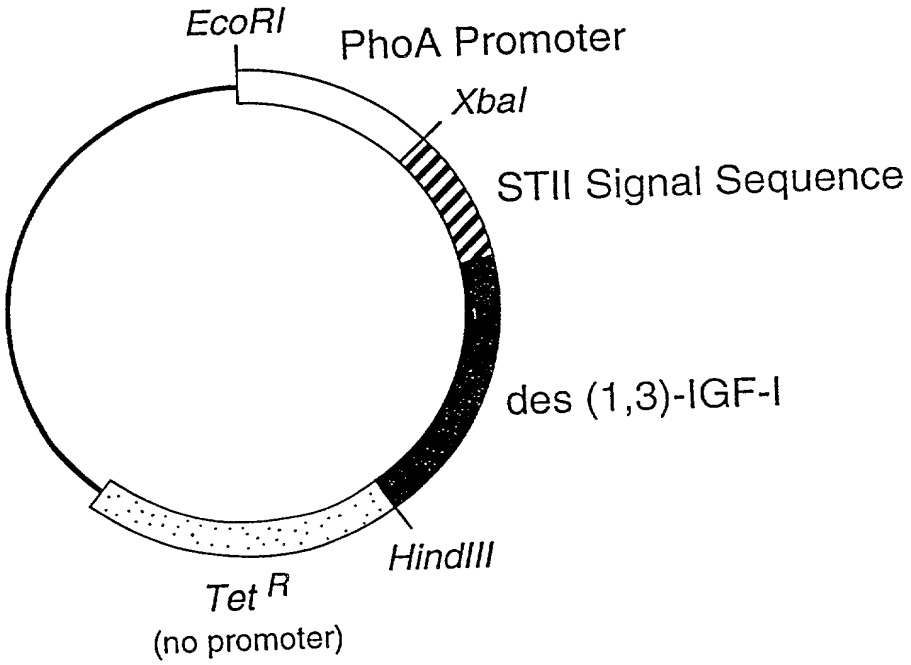

EcoRI
PhoA Promoter
XbaI
STII Signal Sequence
des (1,3)-IGF-I
HindIII
Tet R
(no promoter)

FIG. 2

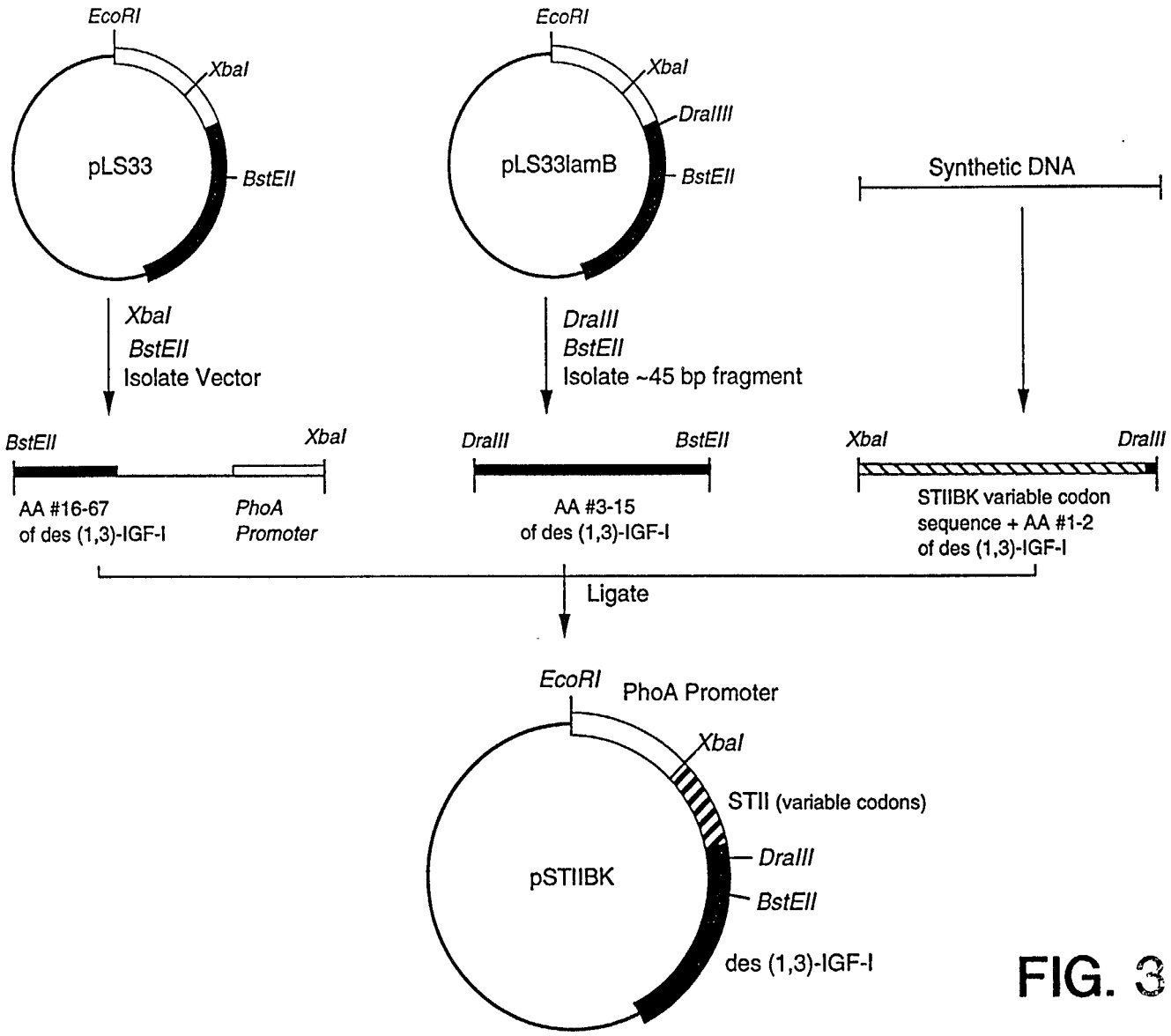

EcoRI
XbaI
pLS33
BstEII
EcoRI
XbaI
DraIII
pLS33lamB
BstEII
Synthetic DNA
XbaI
BstEII
Isolate Vector
DraIII
BstEII
Isolate ~45 bp fragment
BstEII
XbaI
AA #16-67
of des (1,3)-IGF-I
PhoA
Promoter
DraIII
BstEII
AA #3-15
of des (1,3)-IGF-I
XbaI
DraIII
STIIBK variable codon
sequence + AA #1-2
of des (1,3)-IGF-I
Ligate
EcoRI
PhoA Promoter
XbaI
STII (variable codons)
DraIII
pSTIIBK
BstEII
des (1,3)-IGF-I

FIG. 3

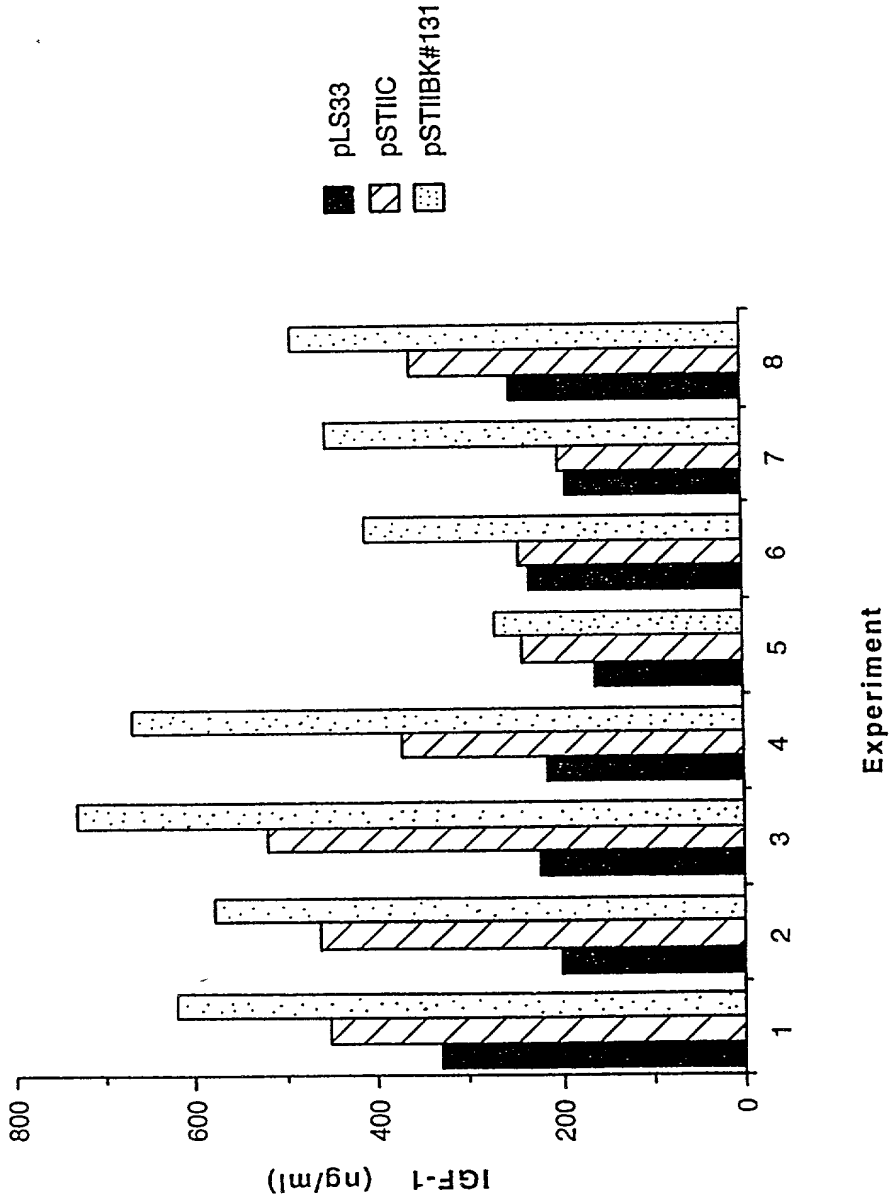

pLS33
pSTIIC
pSTIIBK#131
IGF-1 (ng/ml)
0
200
400
600
800
1
2
3
4
5
6
7
8
Experiment

FIG. 4

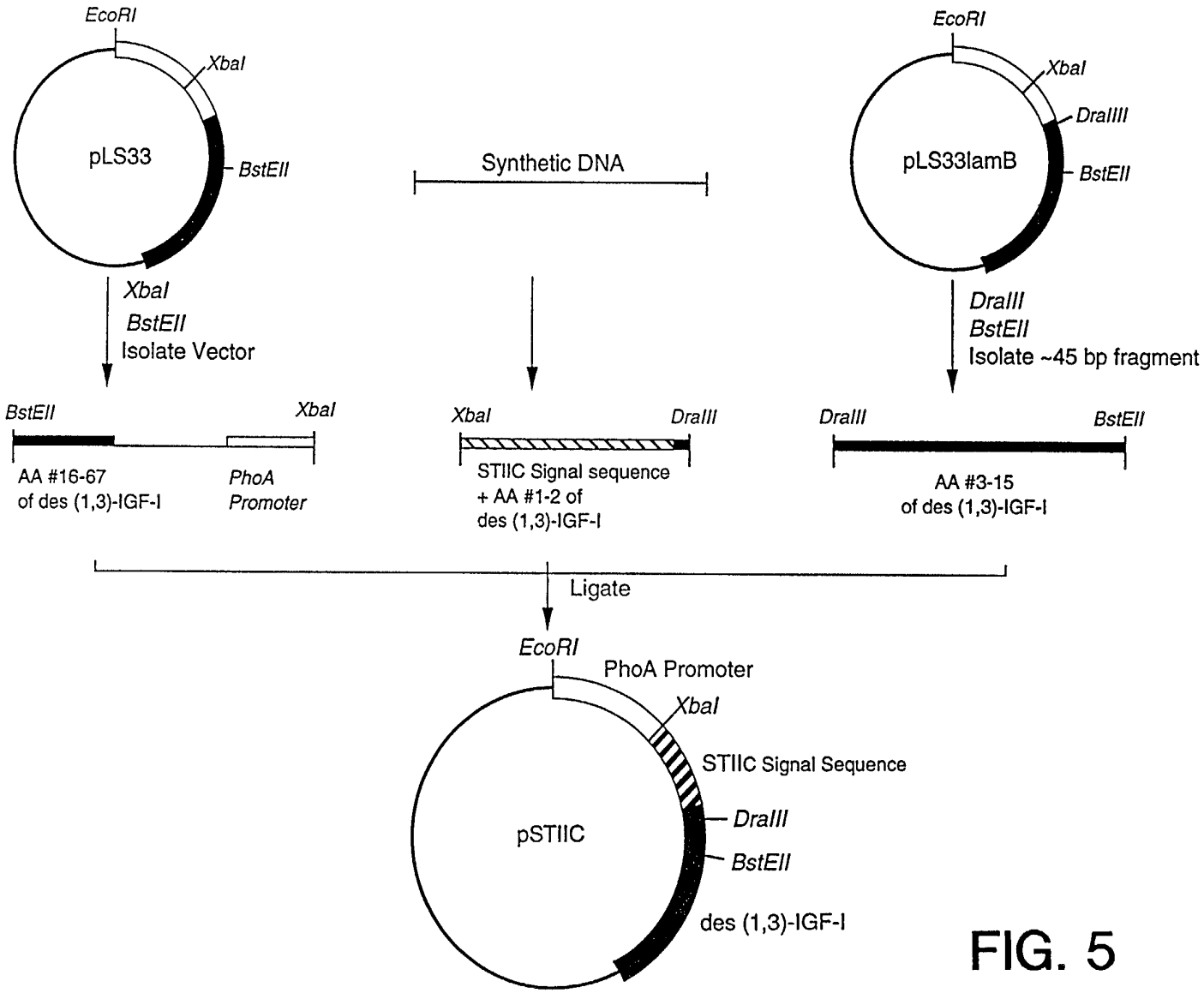

EcoRI
XbaI
pLS33
BstEII
XbaI
BstEII
Isolate Vector
BstEII
XbaI
AA #16-67
of des (1,3)-IGF-I
PhoA
Promoter
Synthetic DNA
XbaI
DraIII
STIIC Signal sequence
+ AA #1-2 of
des (1,3)-IGF-I
EcoRI
XbaI
DraIII
pLS33lamB
BstEII
DraIII
BstEII
Isolate ~45 bp fragment
DraIII
BstEII
AA #3-15
of des (1,3)-IGF-I
Ligate
EcoRI
PhoA Promoter
XbaI
STIIC Signal Sequence
pSTIIC
DraIII
BstEII
des (1,3)-IGF-I

FIG. 5

A-63487-3

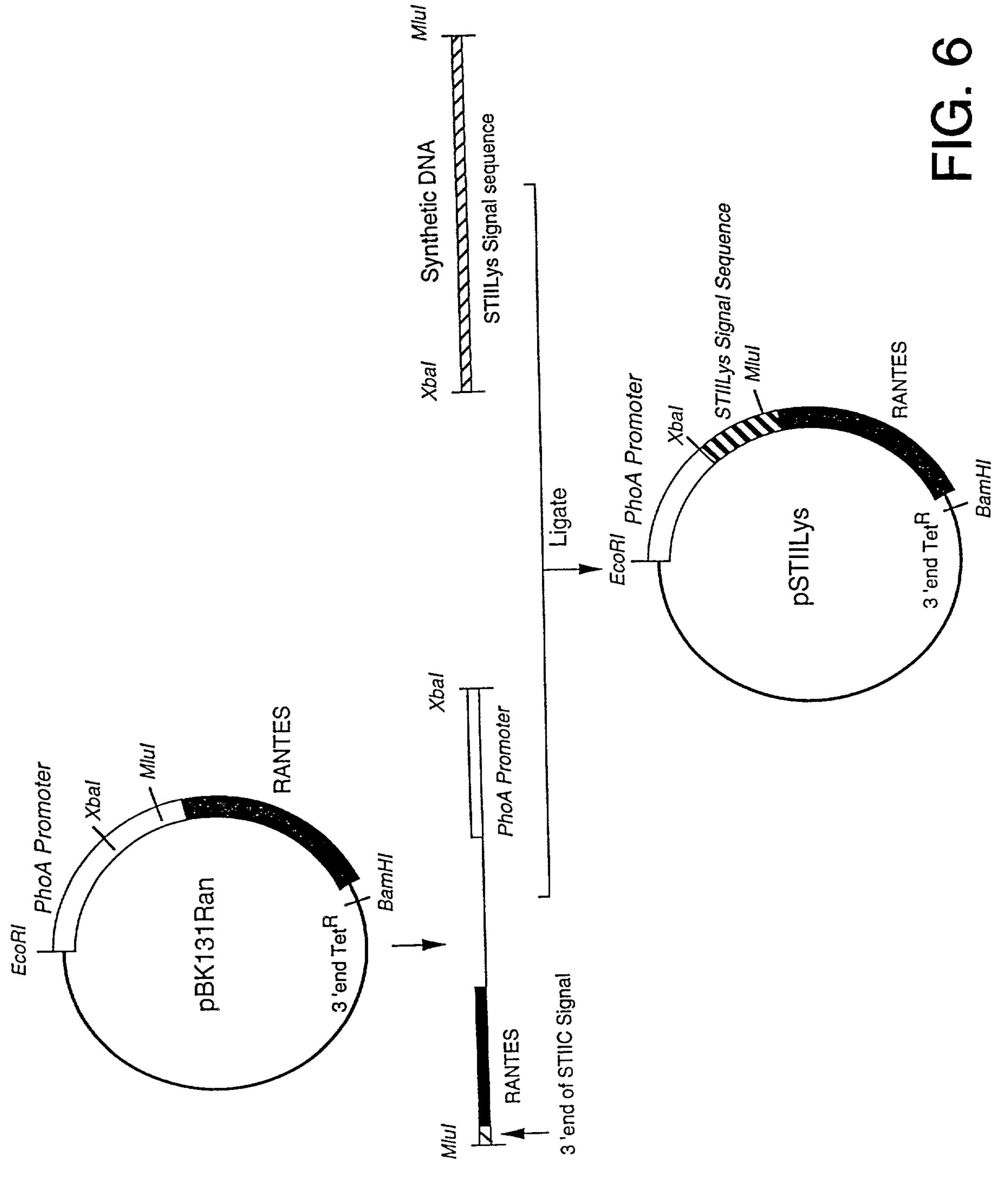


FIG. 6

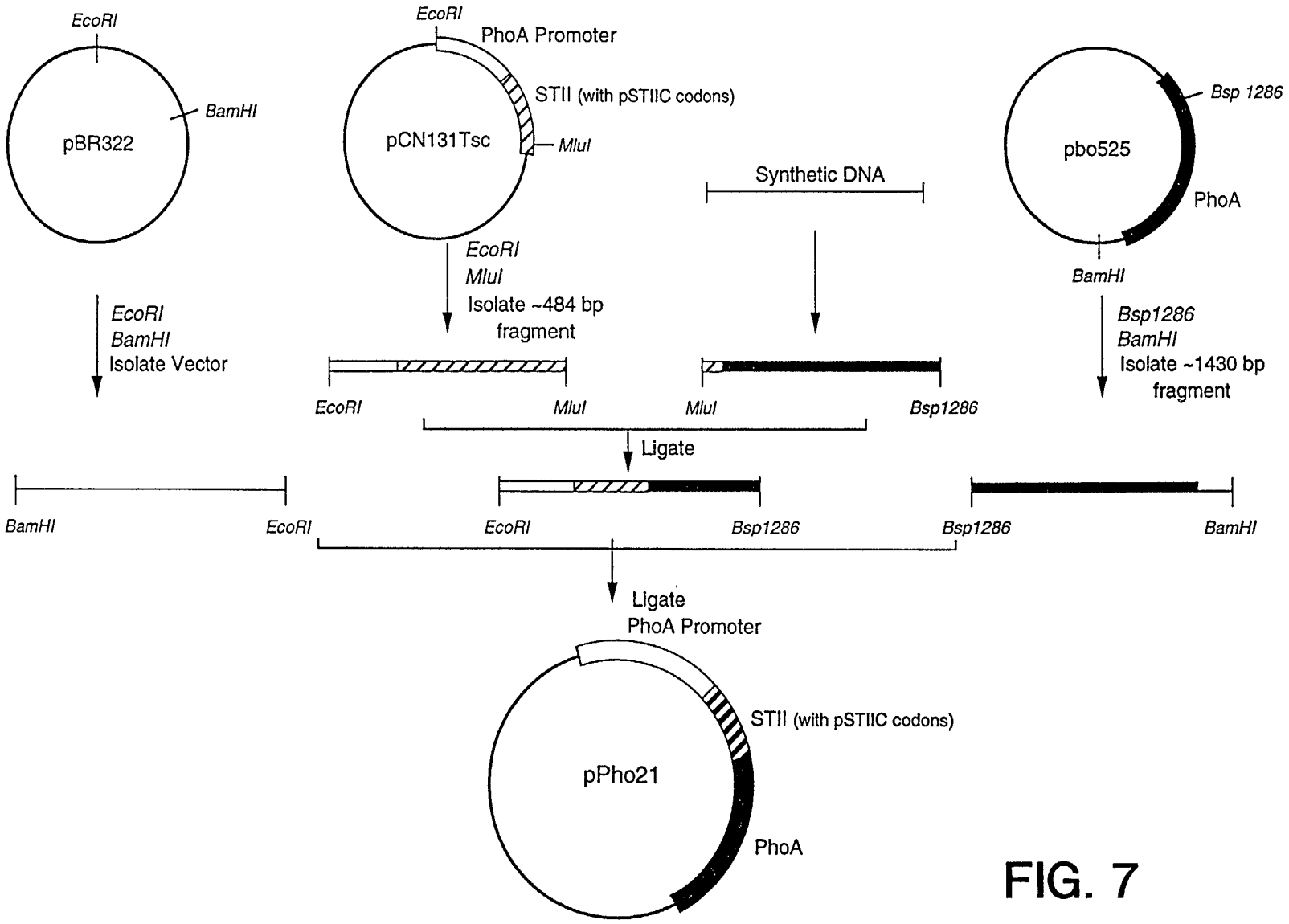


FIG. 7

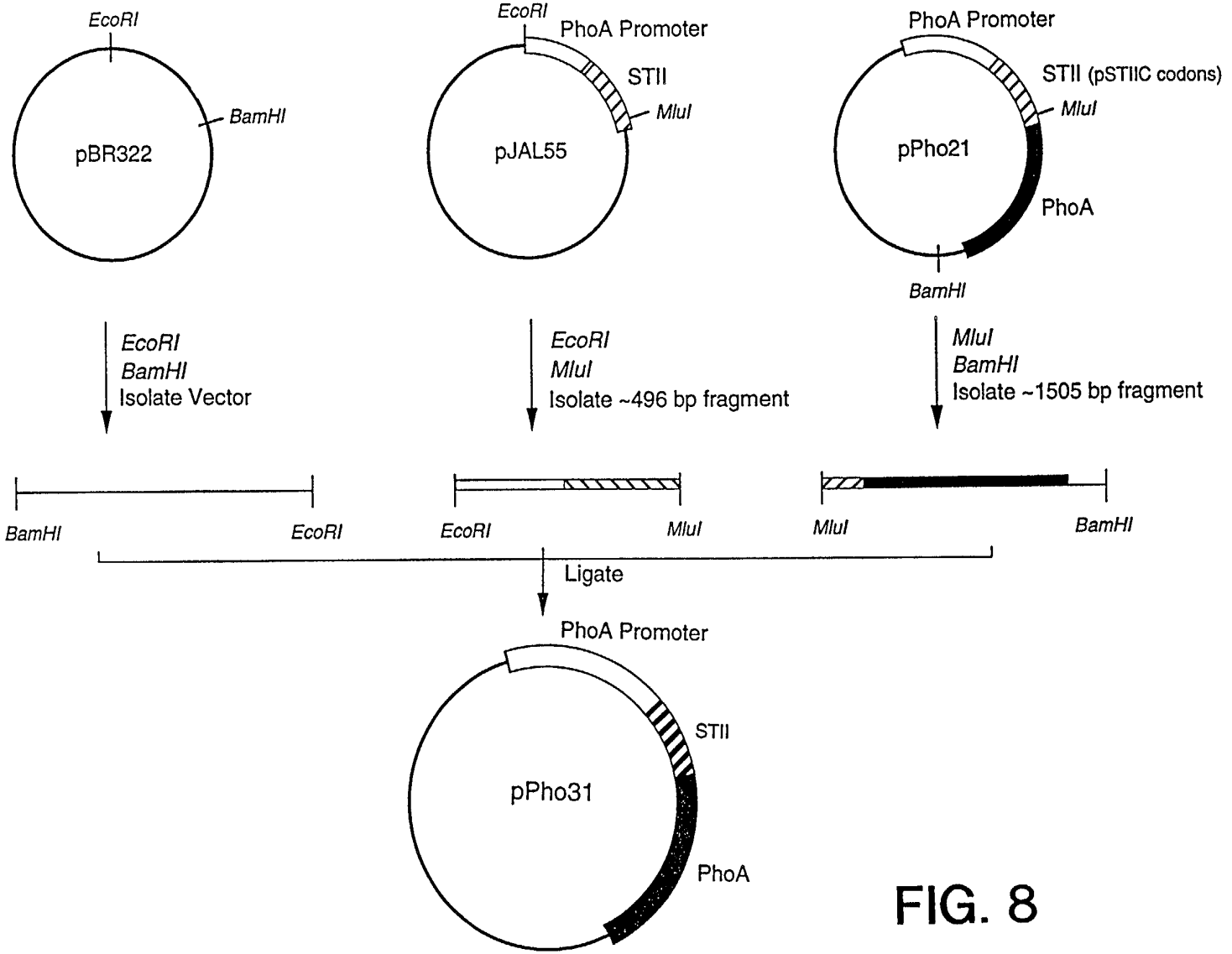

EcoRI
BamHI
pBR322
EcoRI
BamHI
Isolate Vector
BamHI
EcoRI
EcoRI
PhoA Promoter
STII
MluI
pJAL55
EcoRI
MluI
Isolate ~496 bp fragment
EcoRI
MluI
PhoA Promoter
STII (pSTIIC codons)
MluI
pPho21
PhoA
BamHI
MluI
BamHI
Isolate ~1505 bp fragment
MluI
BamHI
Ligate
PhoA Promoter
STII
pPho31
PhoA

FIG. 8

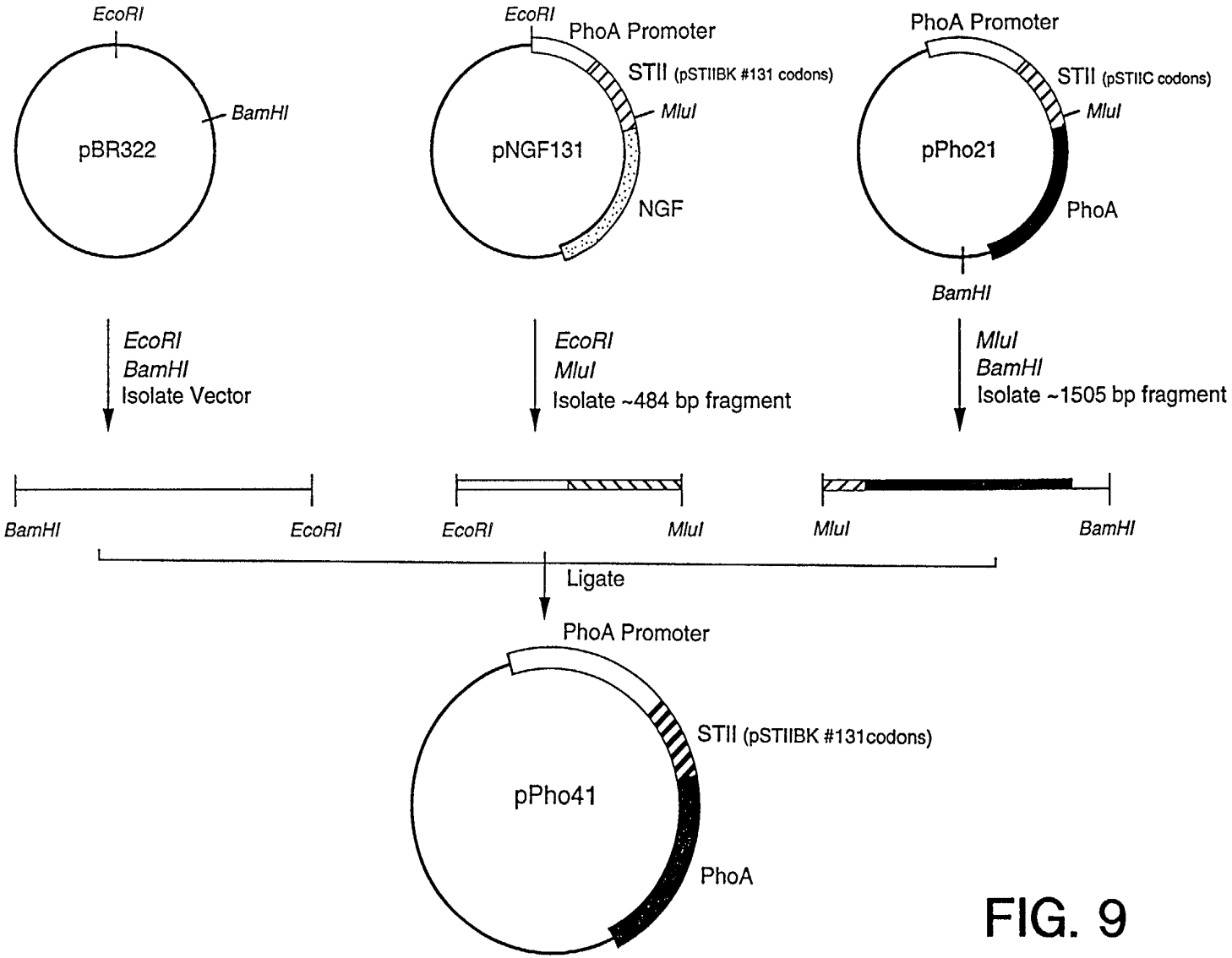


FIG. 9

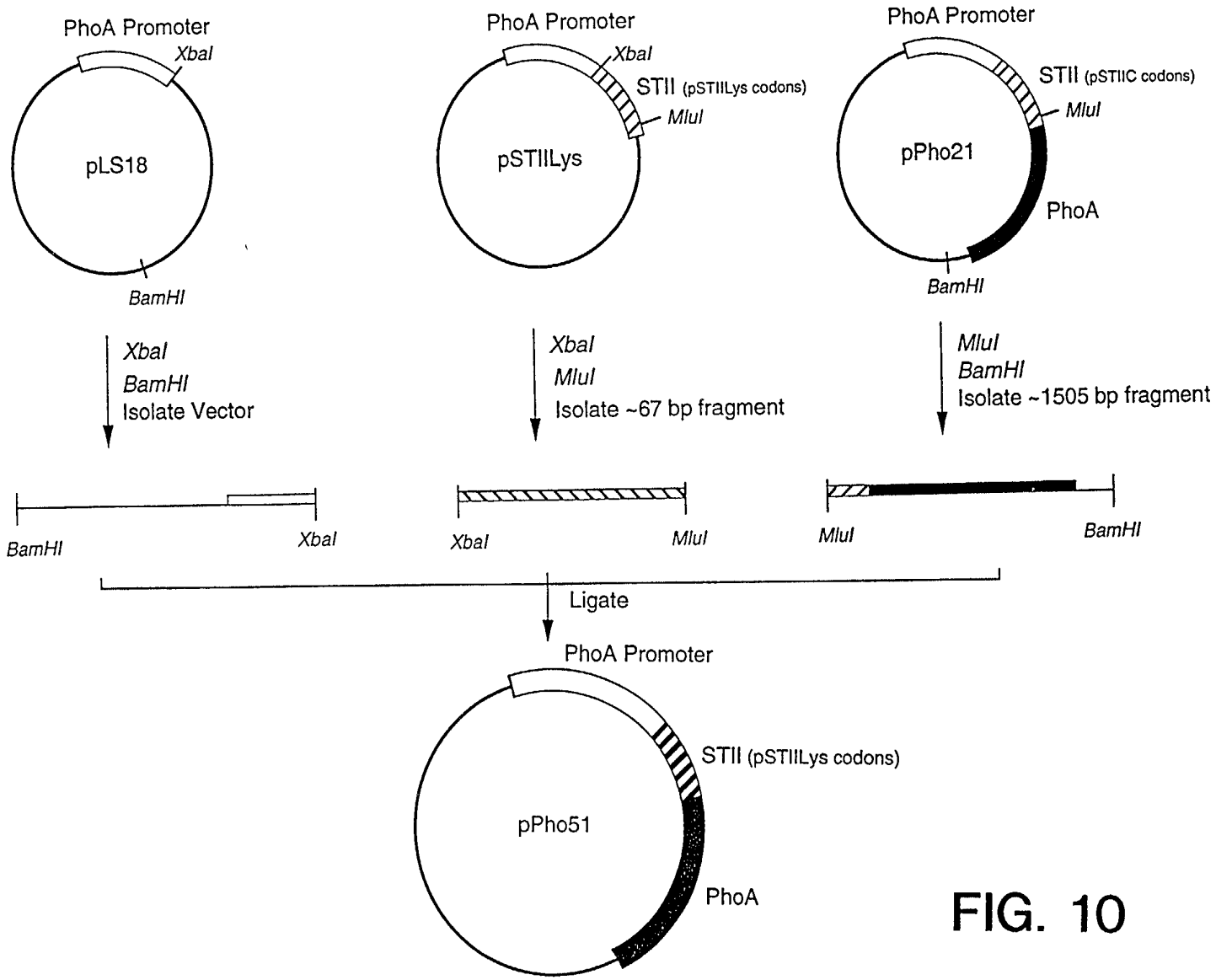

PhoA Promoter
XbaI
pLS18
BamHI
XbaI
BamHI
Isolate Vector
PhoA Promoter
XbaI
STII (pSTIILys codons)
MluI
pSTIILys
XbaI
MluI
Isolate ~67 bp fragment
PhoA Promoter
STII (pSTIIC codons)
MluI
pPho21
PhoA
BamHI
MluI
BamHI
Isolate ~1505 bp fragment
BamHI
XbaI
XbaI
MluI
MluI
BamHI
Ligate
PhoA Promoter
STII (pSTIILys codons)
pPho51
PhoA

FIG. 10

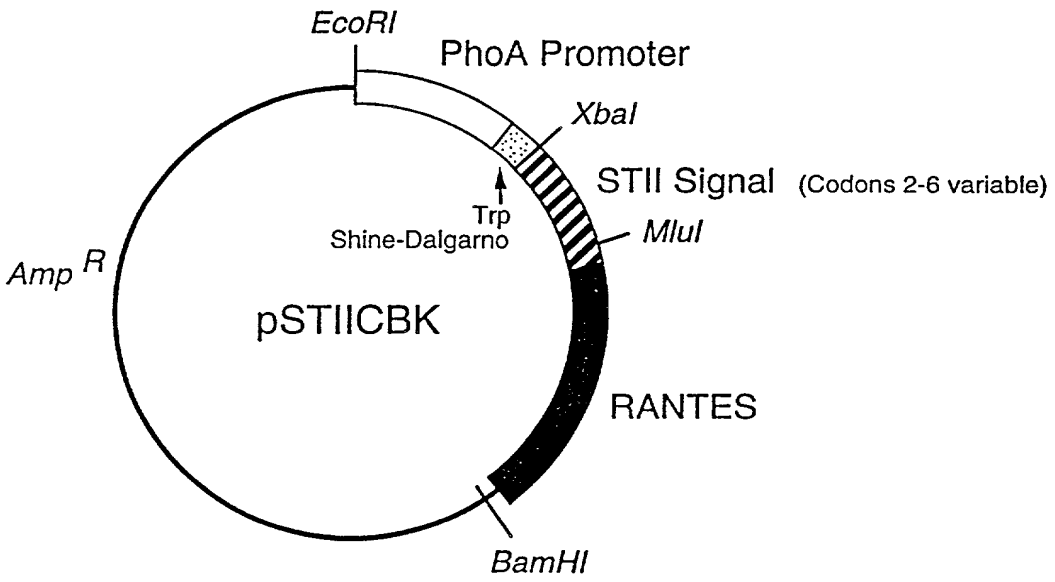

EcoRI
PhoA Promoter
XbaI
STII Signal (Codons 2-6 variable)
Trp
Shine-Dalgarno
MluI
Amp R
pSTIICBK
RANTES
BamHI

FIG. 11

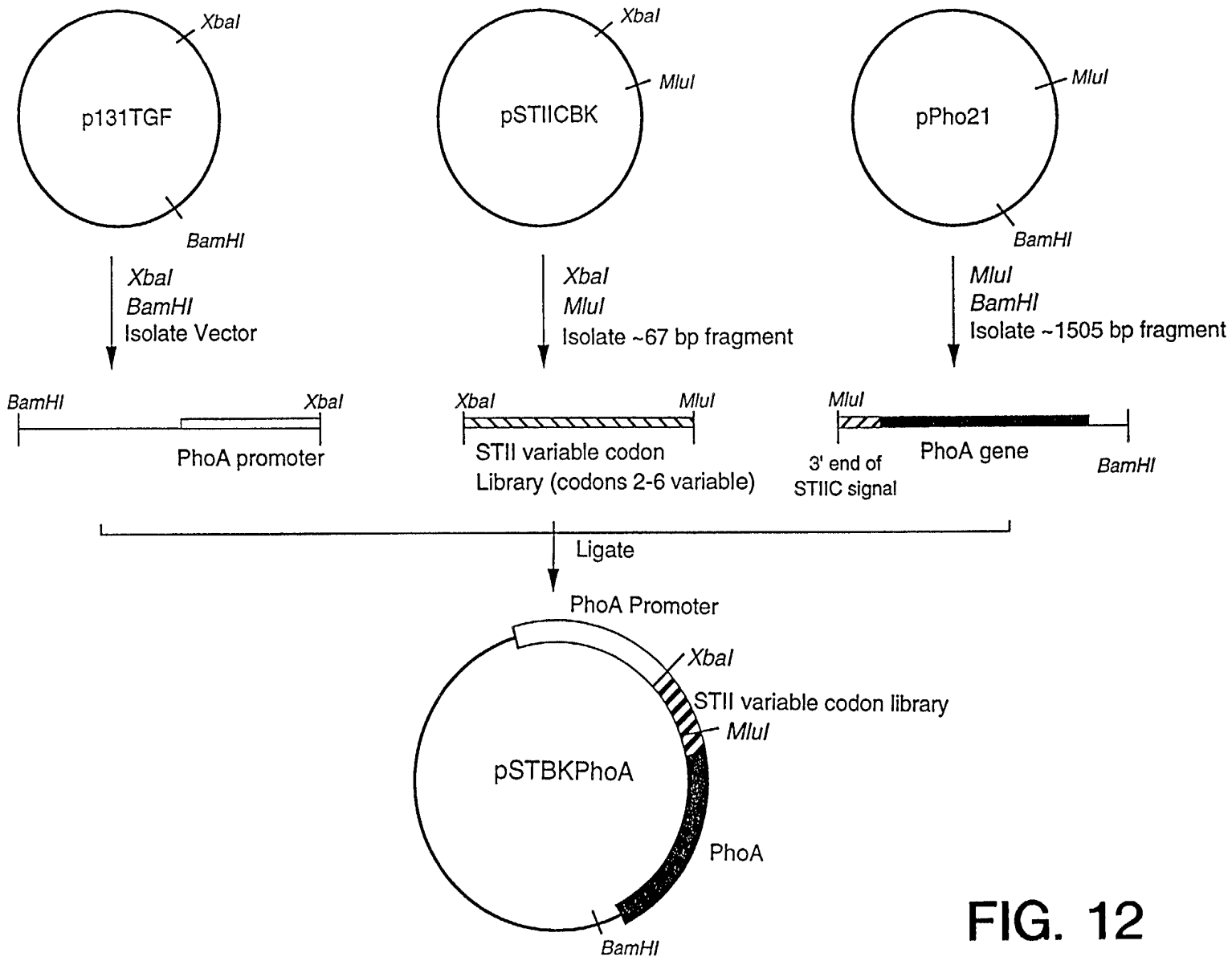


FIG. 12

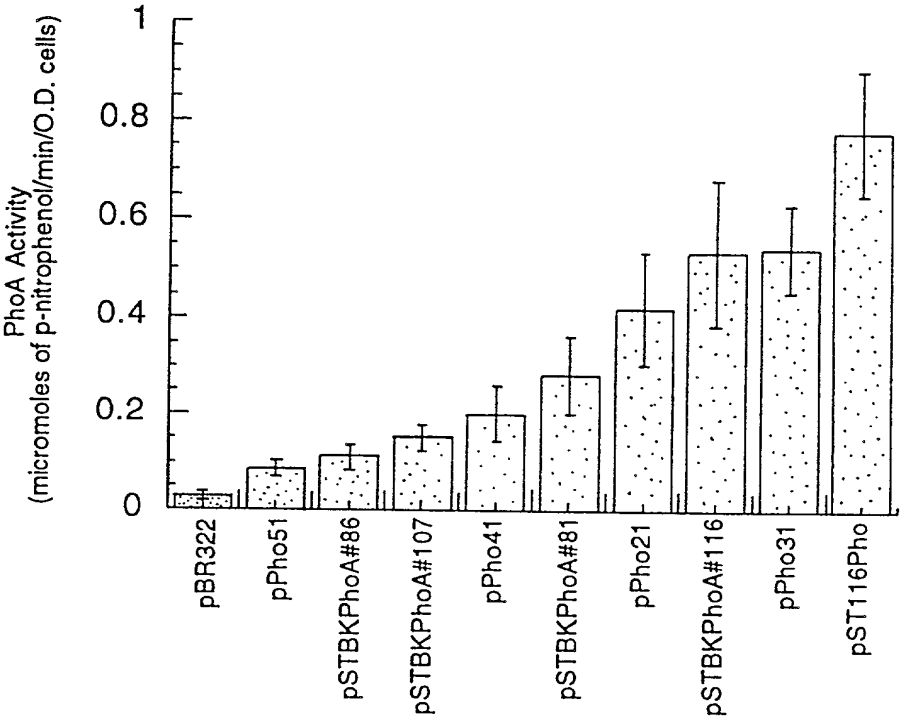

PhoA Activity
(micromoles of p-nitrophenol/min/O.D. cells)
1
0.8
0.6
0.4
0.2
0
pBR322
pPho51
pSTBKPhoA#86
pSTBKPhoA#107
pPho41
pSTBKPhoA#81
pPho21
pSTBKPhoA#116
pPho31
pST116Pho

FIG. 13

pPho31 (Wild type STII + MluI site)
TCTAGAGGTTGAGGTGATTTT ATG AAA AAG AAT ATC GCA TTT CTT CTT GCA TCT ATG TTC GTT

pPho21 (STIIC)
TCTAGAATT ATG AAA AAG AAT ATC GCA TTT CTT CTT GCA TCT ATG TTC GTT

pPho41 (STIIBK#131)
TCTAGAATT ATG AAG AAG AAT ATT GCG TTC CTA CTT GCC TCT ATG TTT GTC

pPho51 (STIILys - unless otherwise noted this sequence is the TIR=1 used in the examples)
TCTAGAATT ATG AAG AAG AAT ATC GCA TTT CTT CTT GCA TCT ATG TTC GTT

pSTBKPhoA#116
TCTAGAATT ATG AAA AAA AAC ATC GCA TTT CTT CTT GCA TCT ATG TTC GTT

pSTBKPhoA#81
TCTAGAATT ATG AAA AAA AAC ATT GCC TTT CTT CTT GCA TCT ATG TTC GTT

pSTBKPhoA#107
TCTAGAATT ATG AAG AAA AAC ATC GCT TTT CTT CTT GCA TCT ATG TTC GTT

pSTBKPhoA#86
TCTAGAATT ATG AAA AAG AAC ATA GCG TTT CTT CTT GCA TCT ATG TTC GTT

pST116Pho
TCTAGAGGTTGAGGTGATTTT ATG AAA AAA AAC ATC GCA TTT CTT CTT GCA TCT ATG TTC GTT

FIG. 14A

		TIR RELATIVE STRENGTH
TTT TCT ATT GCT ACA AAY GCS TAT GCM*	(SEQ ID NO:15)	9
TTT TCT ATT GCT ACA AAC GCG TAT GCM	(SEQ ID NO:16)	7
TTT TCT ATA GCT ACA AAC GCG TAT GCM	(SEQ ID NO:17)	3
TTT TCT ATT GCT ACA AAC GCG TAT GCM	(SEQ ID NO:18)	1
TTT TCT ATT GCT ACA AAC GCG TAT GCM	(SEQ ID NO:19)	9
TTT TCT ATT GCT ACA AAC GCG TAT GCM	(SEQ ID NO:20)	4
TTT TCT ATT GCT ACA AAC GCG TAT GCM	(SEQ ID NO:21)	2
TTT TCT ATT GCT ACA AAC GCG TAT GCM	(SEQ ID NO:22)	1
TTT TCT ATT GCT ACA AAC GCG TAT GCM	(SEQ ID NO:23)	13

* The codons for the last four amino acids of this sequence may differ in some of the examples of protein secretion. For example, in the IGF-1, VEGF165 and RANTES secretion plasmids, the sequence is AAT GCC TAT GCA.
The last codon for the last amino acid in every sequence listed may vary in the examples of protein secretion - GCC and GCA were both used.

FIG. 14B

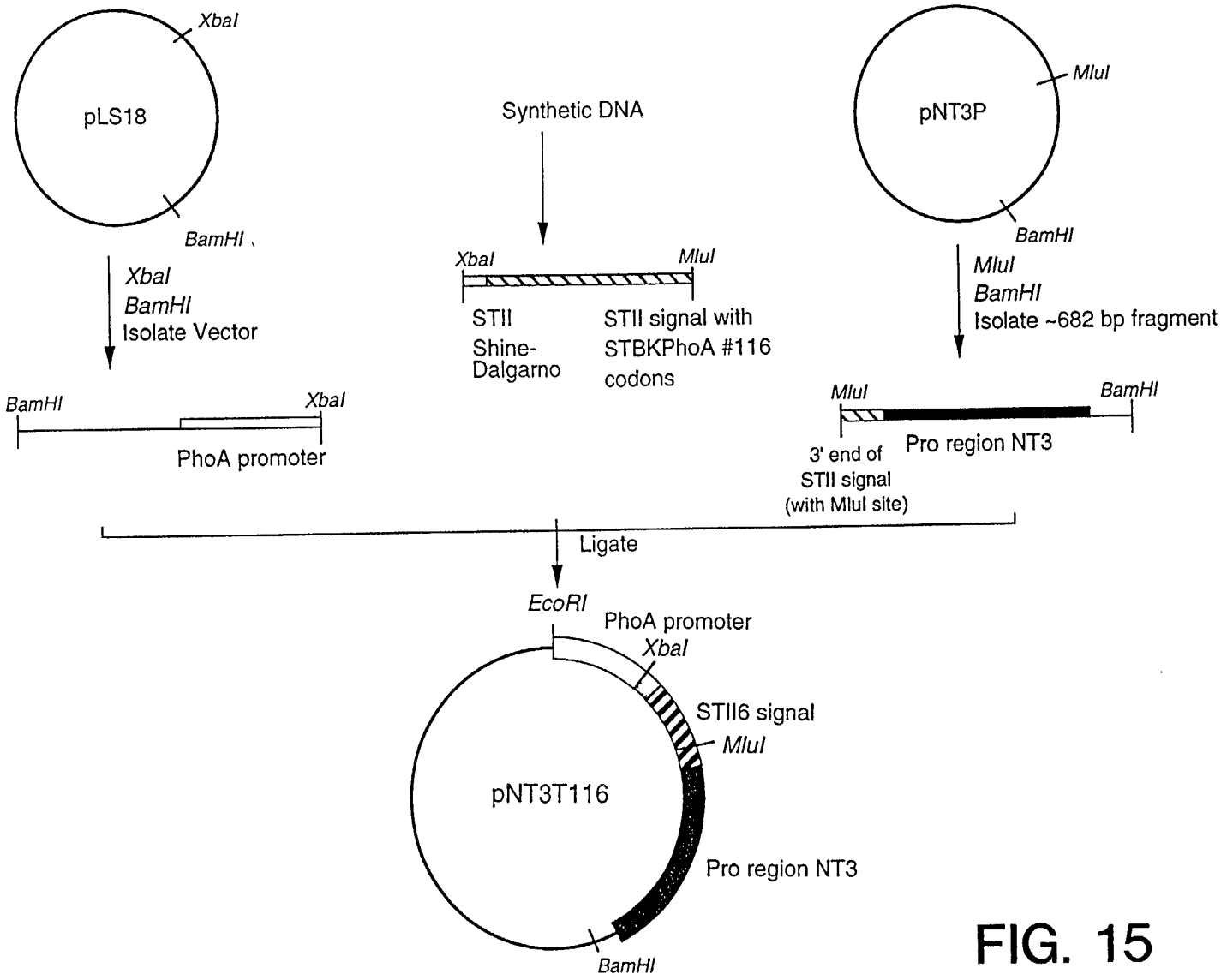

pLS18
XbaI
BamHI
XbaI
BamHI
Isolate Vector
BamHI
XbaI
PhoA promoter
Synthetic DNA
XbaI
MluI
STII
Shine-
Dalgarno
STII signal with
STBKPhoA #116
codons
pNT3P
MluI
BamHI
MluI
BamHI
Isolate ~682 bp fragment
MluI
BamHI
Pro region NT3
3' end of
STII signal
(with MluI site)
Ligate
EcoRI
PhoA promoter
XbaI
STII6 signal
MluI
pNT3T116
Pro region NT3
BamHI

FIG. 15

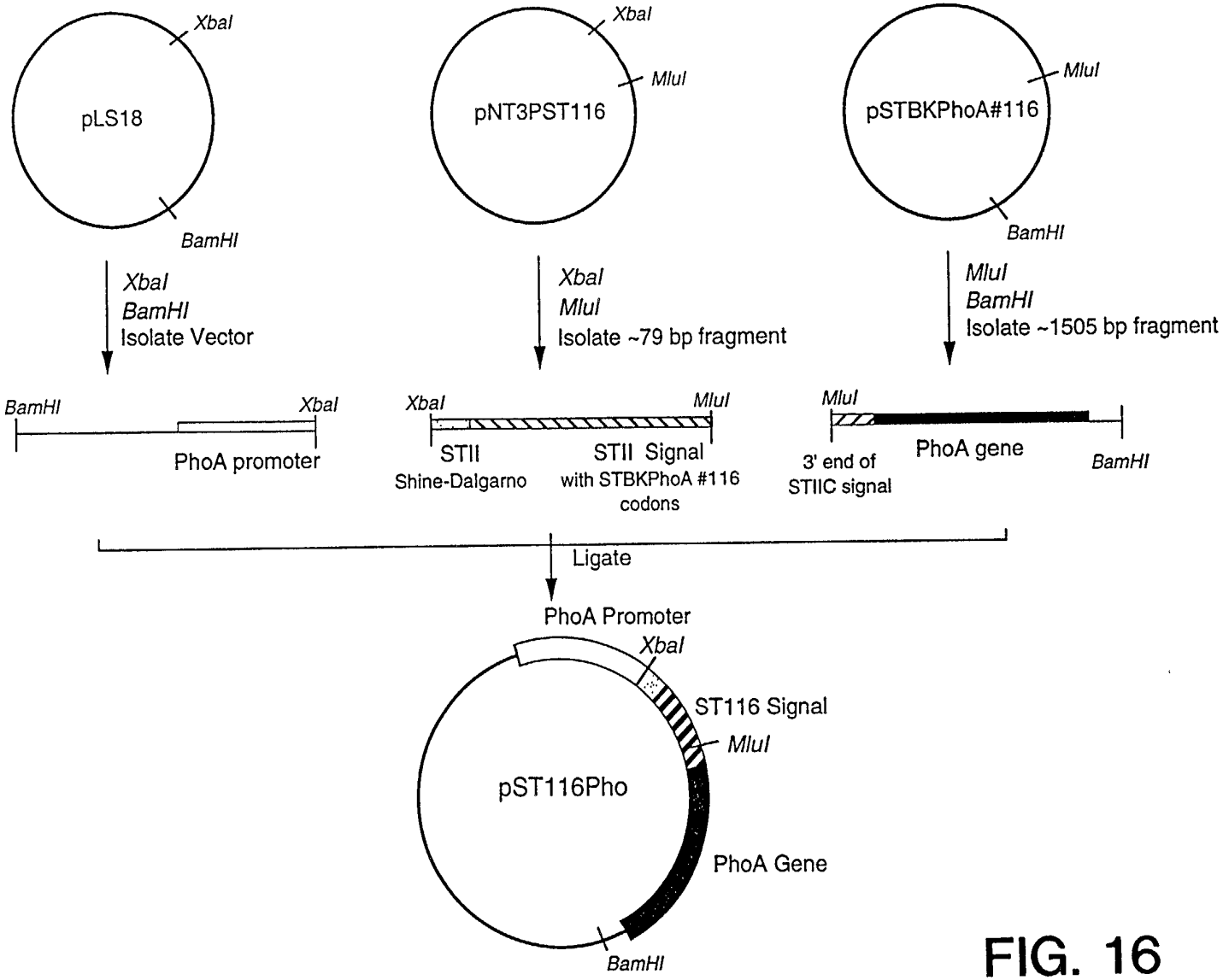
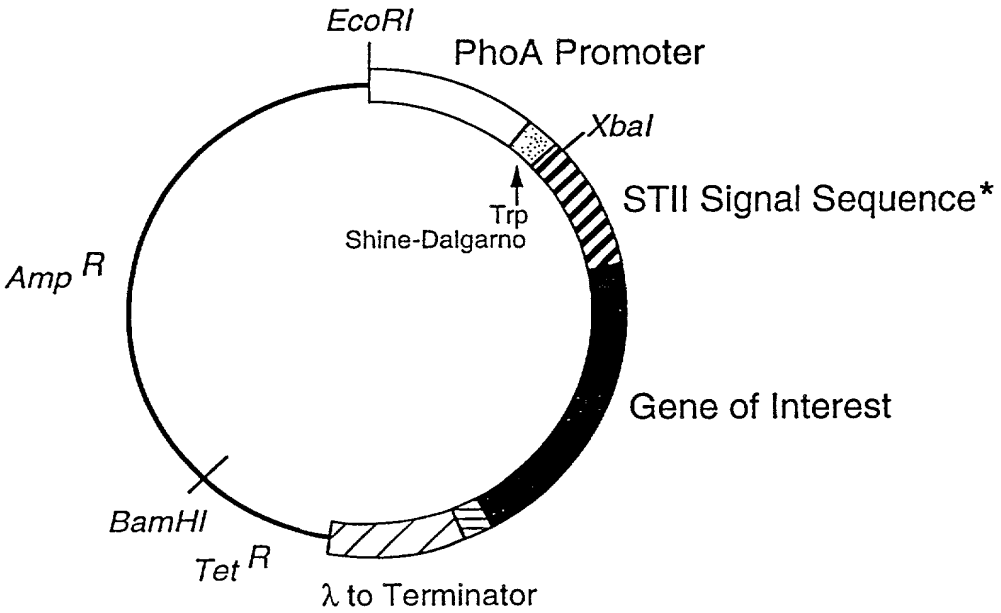
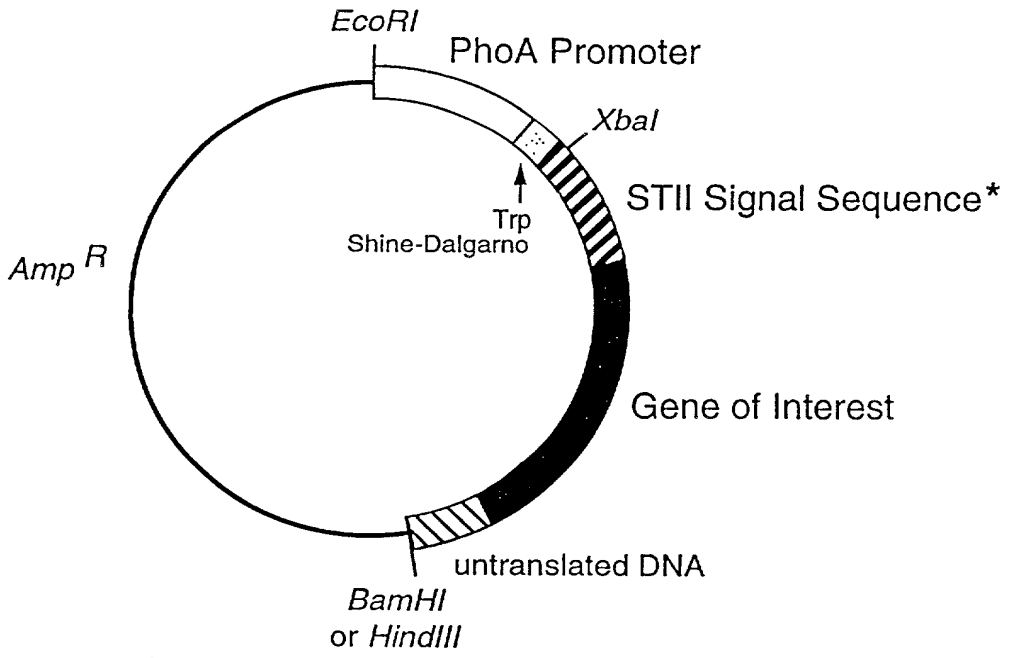

pLS18
XbaI
BamHI
XbaI
BamHI
Isolate Vector
BamHI
XbaI
PhoA promoter
pNT3PST116
XbaI
MluI
XbaI
MluI
Isolate ~79 bp fragment
XbaI
MluI
STII
Shine-Dalgarno
STII Signal
with STBKPhoA #116
codons
pSTBKPhoA#116
MluI
BamHI
MluI
BamHI
Isolate ~1505 bp fragment
MluI
3' end of
STIIC signal
PhoA gene
BamHI
Ligate
PhoA Promoter
XbaI
ST116 Signal
MluI
pST116Pho
PhoA Gene
BamHI

FIG. 16



* One of the nucleotide sequences listed in Figure 14 (STII Shine-Dalgarno may also be included).

FIG. 17



* One of the nucleotide sequences listed in Figure 14 (STII Shine-Dalgarno may also be included).

FIG. 18